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Physicochemical, Nutritional and Sensory Analysis of Sea Buckthorn Drink

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Sea buckthorn (*Hipphophae rhamnoides* L.) is a unique plant, inherent to the Northwest Himalayan region of Pakistan, India, Nepal, and China. It is one of the richest sources of minerals, vitamins, and other nutrients. The fruit is traditionally utilized for the treatment of different diseases in the Northern areas (Gilgit Baltistan) of Pakistan. The present research study has been planned for the evaluation of the nutritional composition of sea buckthorn fruit powder as well as the development of its drink. The sea buckthorn powder was analyzed for nutritional profiling and total phenolic contents. Then, sea buckthorn was utilized to develop a food product (beverage). The product was also analyzed for nutritional and sensory analysis. The results indicated that it contained 5.47±0.09 % moisture, 1.94±0.1% protein, 2.07±0.09% fat, 1.94±0.1% fiber, and 0.48±0.02% ash contents. Microbial analysis of sea buckthorn revealed 3.57±0.45 CFU/g total plate count, while 342.46±3.77 mgGAE/g total phenolic contents. Sea buckthorn drink's physicochemical and organoleptic evaluation showed that it is suitable for commercialization in comparison to other fruit drinks, as well as acceptable to consumers.
Keywords: Sea buckthorn, functional beverage, antioxidant activity, physicochemical properties, sensory evaluation.

INTRODUCTION

Fruits are a rich source of minerals and vitamins, which help and support health and body functions like energy production, breathing, and immune response. Food is primarily consumed as a cell substrate for energy, cell variation and propagation, and as a source of chemical blocks in contradiction of cell oxidation. Foods characterized as functional foods and used for precise dietary purposes (like diet, light, low-fat) are the foremost types studied in the past 20 years. According to Ye et al. (2018), in the 1980's, functional food term was first used in Japan. Though in recent studies, functional foods are defined as natural or technologically processed foods that have possibly positive effects on health beyond basic nutrition, when frequently consumed within a varied diet at an efficient level (Granato et al., 2017).

Functional food is defined by "The Institute of Medicine's Food and Nutrition Board" (IOM/FNB, 1994) as "any food or food ingredient which, beyond the typical nutrients it comprises, provides a health benefit". Functional foods are increasingly associated with mitigating risks of non-communicable diseases such as cardiovascular disorders, diabetes, and cancer (Mak et al., 2018).

According to the knowledge of traditional people and some evidence, the sea buckthorn found in the Himalaya regions in the northern area is traditionally used as food and medicine for animals as well as human beings, since ancient times, but the people are not familiar with scientific backgrounds. Similarly, no techniques are applied for proper processing, preservation and handling of the fruit so that the fruit could be utilized for its therapeutic potentials across the country. On the other hand, the Sea buckthorn oil is not only a source of vegetable oil traditionally produced since ancient times, but is considered to be unique because of the presence of specific carotenoids, fatty acids, and complex lipids. Due to its unique composition, the oil can be used for the treatment of skin diseases, eczema, ulceration, and dermatomes (Bagchi et al., 2014).

Sea buckthorn is a wild, thorny shrub with orange or yellow berries native to the cold, arid areas of the Himalayas and distributed over Pakistan, India, China, and Europe. The berries represent an outstanding source of vitamins, minerals, polyphenols, flavonoids, and essential fatty acids responsible for the high nutraceutical value of this plant (Krejcarova et al., 2015). Sea buckthorn has been used in the traditional medicine of many countries, particularly in the Himalayan and Central Asian regions, for treating ulcers, skin infections,

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cardiovascular diseases, and inflammation (Bagchi et al., 2014).

Sea buckthorn plants grow under extreme climatic conditions, from -40°C to 40°C, showing resistance to drought and poor soil conditions, while their roots help in the stabilization of the soil and nitrogen fixation (Kumar, 2003). Due to its ecological adaptability, the plant is considered one of the important species for reforestation and rehabilitation of mountainous and semi-arid lands. Morphologically, the berries possess pulp and seed fractions, both of which are rich in different bioactive components. The pulp contains high amounts of carotenoids, vitamin C, and polyunsaturated fatty acids, while the seed oil is rich in essential fatty acids like linoleic and linolenic acids, tocopherols, and phytosterols, which are well known for their antioxidative and cardioprotective properties (Mortensen et al., 2018; Zhao et al., 2017). These compounds act synergistically to neutralize reactive oxygen species, enhance immune defense, and prevent oxidative damage associated with degenerative diseases. Therefore, Guo et al. (2017) state that the high levels of total phenolic compounds and flavonoids position sea buckthorn as an ideal candidate for functional food formulation targeting health conditions related to oxidative stress.

Functional beverages fortified with bioactive ingredients are considered convenient vehicles for the delivery of nutrients and health-promoting compounds worldwide. Functional drinks made with plant extracts, fruits, and probiotics offer easily accessible alternatives to improve consumers' health. Due to its special phytochemical profile, sea buckthorn is considered a very promising base ingredient for developing such beverages. Bioactive polysaccharides and prebiotic compounds present in it have been demonstrated to enhance probiotic growth and viability, hence increasing its health potential when incorporated into nutraceutical drinks (Gunenc et al., 2016; Gibson et al., 2017).

The present study was conducted to evaluate the nutritional profile of Sea buckthorn berries (*Hippophae rhamnoides* L.) from Northern Pakistan, formulate a value-added sea buckthorn-based drink as a functional beverage, and determine its physicochemical and sensory quality characteristics. This study contributes to academic knowledge, as well as practical applications in food product development, by providing scientific data on the nutritional and sensory profile of sea buckthorn drinks.

MATERIALS AND METHODS

***Sample Collection*:** The fruit of Sea buckthorn (*Hippophae rhamnoides* L.) was collected from Gilgit Baltistan, Pakistan. The equipment and chemicals used for this study will be provided by the Department of Food Science and Technology, MNS- University of Agriculture, Multan.

***Preparation of Sea Buckthorn Powder*:** The collected berries were washed, and the seeds were removed. Then the fruit was sun-dried, powdered, and stored in airtight containers at room temperature for further use in beverage formulation.

***Development of Sea Buckthorn Drink*:** Sea buckthorn (*H. rhamnoides*) powder was obtained and used for the development of a beverage and sodium benzoate was used as a preservative. The drink/beverage thus prepared, pasteurized at 85-95°C for 15-30 seconds, and shelf stability was studied at room temperature. The fruit beverage was analyzed for different quality and sensory attributes.

Table 1. Recipe used for Sea Buckthorn Drink Development.

Ingredients	Quantity
Water	1000 mL
Sea buckthorn powder	300 g
Sugar	200 g
Citric Acid	2 g
Sodium Benzoate	1 g

***Proximate Analysis*:** Proximate composition (moisture, protein, fat, fiber, and ash content) of the sea buckthorn powder and formulated drinks was determined according to the AOAC (2019) methods.

All analyses were conducted in triplicate, and results were expressed as mean ± standard deviation on a dry-weight basis.

Physicochemical Analysis: The physicochemical parameters, including pH, total soluble solids, titratable acidity, and acidity/brix ratio, of the developed drinks were analyzed using the AOAC (2019) standard methods.

***pH**:* pH was measured using a digital pH meter (Model: Hanna HI 2211).

***Total soluble solids (°Brix)**:* Total soluble solids (°Brix) were determined using a digital hand refractometer.

***Titratable acidity**:* Titratable acidity was calculated by titrating the sample against 0.1 N NaOH using phenolphthalein as an indicator, and the results were expressed as a percentage of citric acid.

***Acidity/Brix ratio**:* The Acidity/Brix ratio was derived from the corresponding values of acidity and °Brix.

***Mineral profile*:** The minerals in the Sea buckthorn drink were determined using an atomic absorption spectrophotometer and a Flame photometer-410 according to the AOAC (2019) criteria. A portion of the sample (1 g) was digested in the nitric and perchloric acid mixture (7 and 3 mL, respectively) until colour and odour disappeared. Then the solution was diluted to 100 mL with distilled water. The standard solutions of calcium, potassium, and sodium were made for analysis on a flame photometer. Wet digestion with nitric, sulphuric and perchloric acids in an 11:2:5 ratio, respectively, was carried out for metals like iron and zinc. One gram of the sample was digested in nitric acid (10 mL)

followed by the addition of 60 % $HClO_4$ (3 mL) and gently heated until the solution evaporated to 0.5-1.0 mL.

Microbial stability evaluation: The product was subjected to microbial examination, including total plate count, yeast and mold count, during the storage period of the drink according to the methods given by Harrigan (1998). The agar medium was used for microbial stability evaluation. The agar medium was sterilized in an autoclave at 121^0C for 15 minutes.

$$\text{Viable count } \left(\frac{cfu}{ml}\right) = \frac{Number\ of\ colonies * Dilution\ factor}{Volume\ plated\ (mL)}$$

Total phenolic contents (TPC): Total phenolic contents of sea buckthorn fruit powder drink were determined using the Folin-Ciocalteu's reagent, followed by the method which is prescribed by Singleton et al. (1999). For this purpose, accurately 70 µL of prepared buckthorn drink sample was added in a test tube of 10 mL capacity, followed by 250 µL of Folin-Ciocalteu reagent and 750 µL of Na_2CO_3 (1.9 M). However, a total volume of exactly 5 ml was made up by adding distilled water, then mixed by using a Vortex mixer for about 1 minute before incubation for 2 hours in the dark. Consequently, the absorbance was measured by utilizing a Spectrophotometer (Thermo-Spectronic, Surrey, England) at 765 nm wavelength. A calibration curve was prepared by employing controlled solutions of Gallic acid. Total polyphenols determined and expressed as gallic acid equivalent (mg gallic acid/g).

$$TPC\ (mg\ GAE/g) = \frac{GAE\ \left(\frac{mg}{L}\right)[total\ volume\ of\ the\ methanol] \times dilution\ factor}{Sample\ weight\ (g)}$$

Sensory assessment: Nine hedonic measures were used to assess the sensory evaluation of sea buckthorn drink, including taste, flavor, color, aroma, texture, appearance, and overall consumers' acceptability and suitability (Meilgarard et al., 2007). 15 highly qualified researchers looked into the sensory characteristics of sea buckthorn drink. Sensory Performa sheet was given to each researcher & asked them to scale the samples according to color, taste, texture, aroma, and overall acceptability. These attributes scored at a respectable level.

Storage study: The final product was stored at room temperature for 3 months, and analyses were conducted at 0, 15, 30, 45, 60, 75, and 90 days of the storage period

***Statistical analysis*:** The obtained data from research were subjected for the ANOVA(ANOVA) by the use of STATISTIX (Version 8.1) software (Montgomery, 2017).

RESULTS

Proximate analysis of Sea buckthorn powder: Proximate composition of Sea buckthorn berry powder was evaluated, and results showed that 100 g of berry powder contains moisture (5.47±0.09), crude fat (2.07±0.09), and crude protein (1.94±0.1). The Sea buckthorn berry powder showed crude fiber (0.48±0.02**),** ash content (0.48±0.02), calcium (53.70±0.85), sodium (194.33±2.33), potassium (75.50±1.55), zinc (207.00±1.15), and magnesium (30.40±0.21). The result of total phenols and total plate count in Sea buckthorn powder was observed as 342.46±3.77mgGAE/g and 3.57±0.45 CFU/g, respectively

Table 2. Mean values of proximate composition of Sea buckthorn berry powder.

Parameters	Values
Moisture (%)	5.47±0.09
Crude protein (%)	1.94±0.10
Crude fat (%)	2.07±0.09
Crude Fiber (%)	0.48±0.02
Ash (%)	0.48±0.02
Total phenolic (mgGAE/g)	342.46±3.77
Calcium (mg)	53.70±0.85
Total plate count (cfu/g)	3.57±0.45
Potassium (mg)	75.50±1.55
Zinc (mg)	207.00±1.15
Magnesium (mg)	30.40±0.21
Sodium (mg)	194.33±2.33

Physical and chemical analysis of Sea buckthorn drink

pH: The result further indicates that the pH of Sea buckthorn juice significantly declined during storage, the highest pH juice was found at zero day and 15th day in storage, with mean values of 3.42±0.009 and 3.37±0.017. Whereas, the lowest pH of juice was examined at the 90th day and 75th day of storage with the mean of 3.01± 0.015 and 3.10±0.035, as shown in Figure 1.

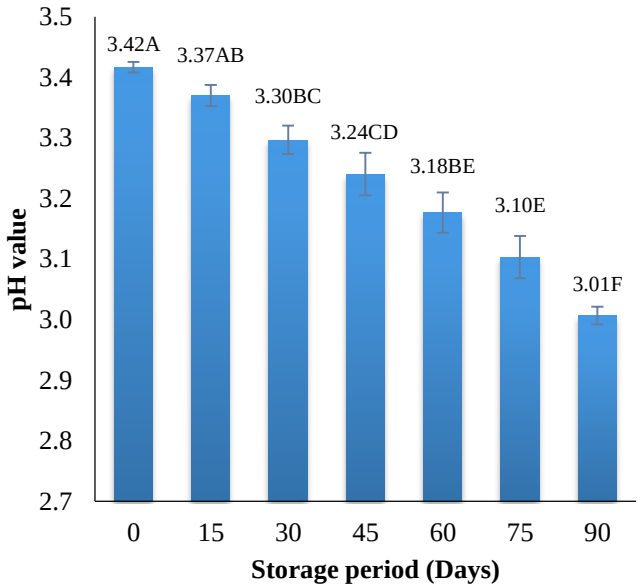

Figure 1. Storage effect on the pH of the sea buckthorn drink.

Brix: The results showed that the Brix of Sea buckthorn was increased significantly during storage, the lowest Brix juice was found at zero day and 15th day in storage with mean values of 11.07± 0 .033and 11.11±0.007. Whereas, the highest Brix/Acidity of juice was examined at the 90th day and 75th day of storage with the mean of 11.98± 0.079and 11.82±0.052, as shown in Figure 2.

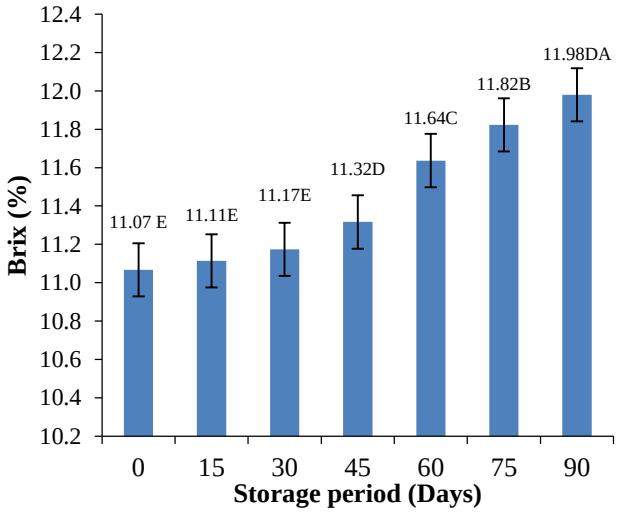

Figure 2. Storage effect on the Brix (%) of sea buckthorn drink.

Acidity/brix ratio: The effect further indicate that Acidity/Brix of Sea buckthorn was increased during storage significantly, the highest Acidity/Brix juice was found at 75 and 90 days in storage with mean value of 6.02±0.053 and 6.32± 0.067 Whereas, the lowest Acidity/Brix of juice was observed at zero and 5th day of storage with the mean of 4.79±0.030 and 4.86±0.009 as show in Figure 3.

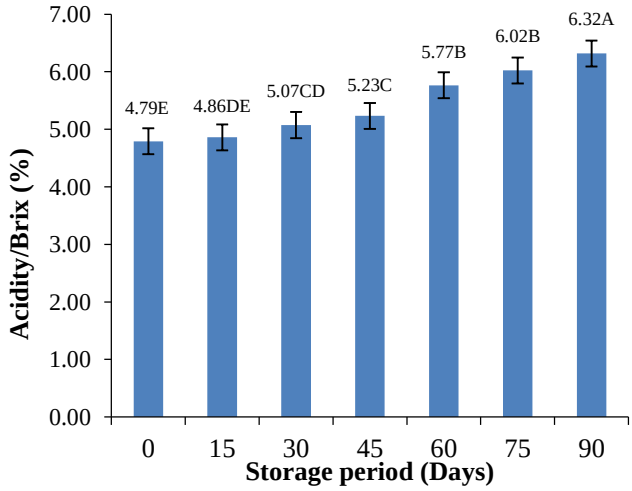

Figure 3. Storage effect on the Acidity/Brix ratio of sea buckthorn drink.

Mineral Analysis

Sodium: The results indicate that the sodium of Sea buckthorn declined during storage, the highest sodium content was found at zero day and 15th day in storage, with mean values of 51.60±0.208 and 50.53±0.464. Whereas, the lowest sodium of juice was examined at the 90th day and 75th day of storage with the mean of 46.87± 0.145 and 47.60±0.153, as shown in Figure 4.

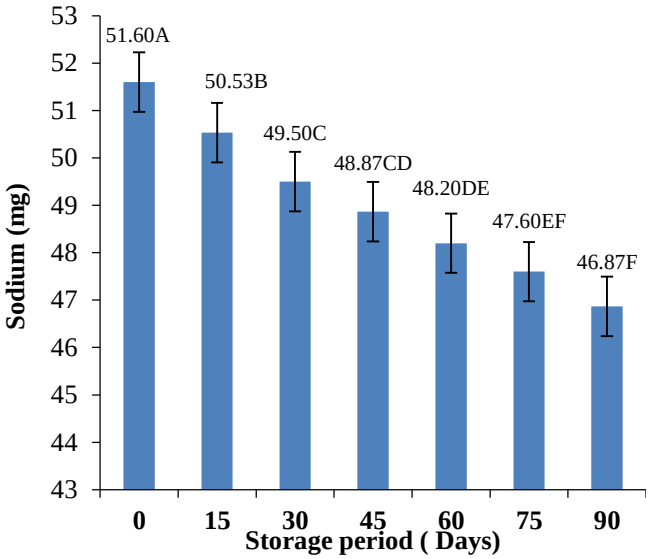

Figure 4. Storage effect on the Sodium contents of sea buckthorn drink.

Calcium: The results indicate that the Calcium of Sea buckthorn declined during storage, the highest Calcium juice was found at zero day and 15th day in storage with mean values of 11.03±0.145 and 10.63±0.088. Whereas, the lowest Calcium of juice was examined at the 90th day and 75th day of storage with the mean of 8.77±0.177 and 9.10±0.153, as shown in Figure 5.

Potassium: The results indicate that the potassium of Sea buckthorn declined during storage, the highest potassium juice was found at zero day and 15th day in storage, with mean values of 62.67±0.441 and 62.13±0.334. Whereas, the lowest potassium of juice was examined at the 90th day and 75th day of storage with the mean of 59.27±0.471 and 59.83±0.485, as shown in Figure 6.

Sensory Analysis

Color: The result indicates that the color of Sea buckthorn declined during storage, the highest color juice was found at zero day and 15th day in storage, with mean values of 8.97±0.006 and 8.83±0.004. Whereas, the lowest color of juice was examined at the 90th day and 75th day of storage with the mean of 7.72±0.008 and 7.99±0.005, as shown in Figure 7.

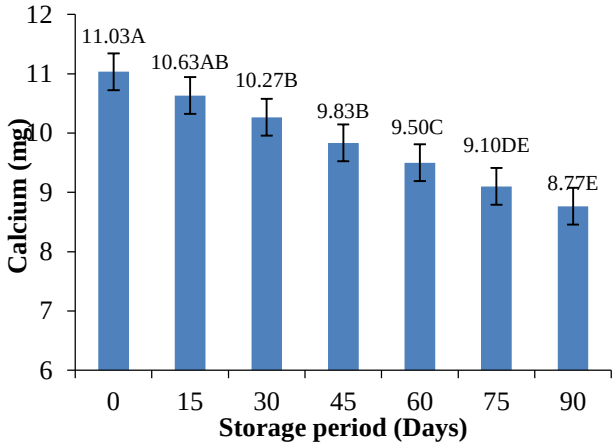

Figure 5. Storage effect on the Calcium contents of sea buckthorn drink.

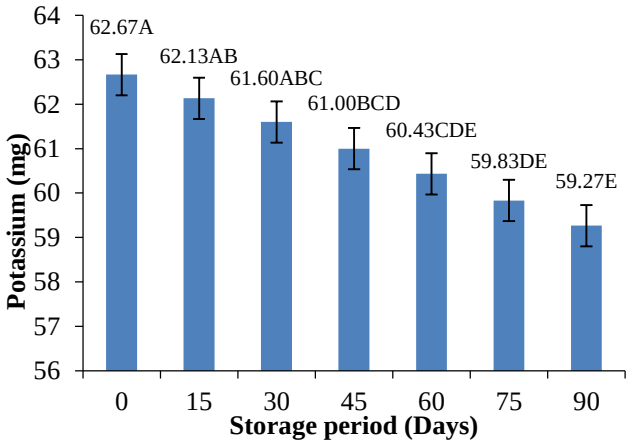

Figure 6. Storage effect on the potassium contents of sea buckthorn drink.

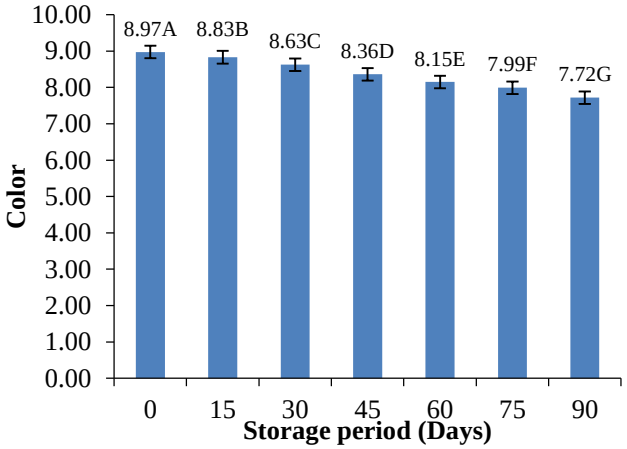

Figure 7. Storage effect on the color of the sea buckthorn drink.

Flavor: The results indicate that the flavor of Sea buckthorn declined during storage, the highest flavor juice was found at zero day and 15th day in storage with mean values of 8.91±0.017 and 8.81±0.005. Whereas, the lowest flavor of drink was examined at the 90th day and 75th day of storage with the mean of 7.19± 0.047 and 8.04±0.030, as shown in Figure 8.

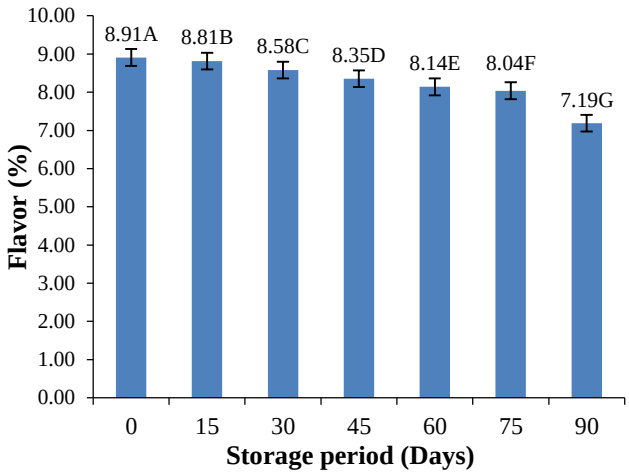

Figure 8. Storage effect on the flavor of sea buckthorn drink.

Taste: The results indicate that the taste of Sea buckthorn declined during storage, the highest taste juice was found at zero day and 15th day in storage with mean values of 8.98±0.006 and 8.83±0.004. Whereas, the lowest taste of juice was examined at the 90th day and 75th day of storage with the mean of 7.72± 0.008 and 8.00±0.005, as shown in Figure 9.

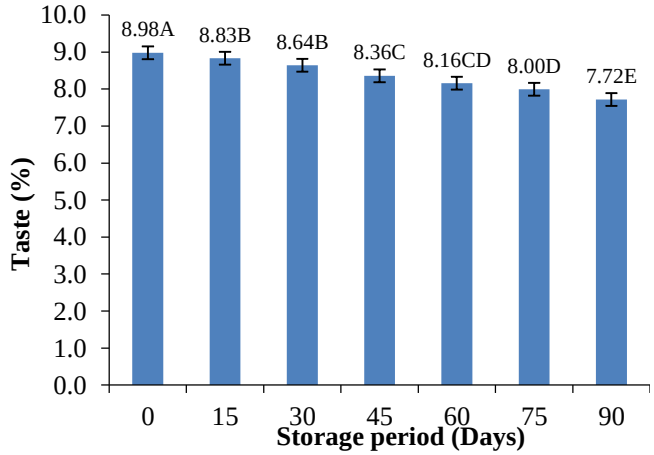

Figure 9. Storage effect on the taste of sea buckthorn drink.

***After Taste*:** The results indicate that the after taste of Sea buckthorn declined during storage, the highest after taste juice was found at zero day and 15th day in storage with mean values of 8.95±0.007 and 8.82±0.006. Whereas, the lowest aftertaste of juice was examined at the 90th day and 75th day of storage with the mean of 7.15±0.011and 7.97±0.012, as shown in Figure 10.

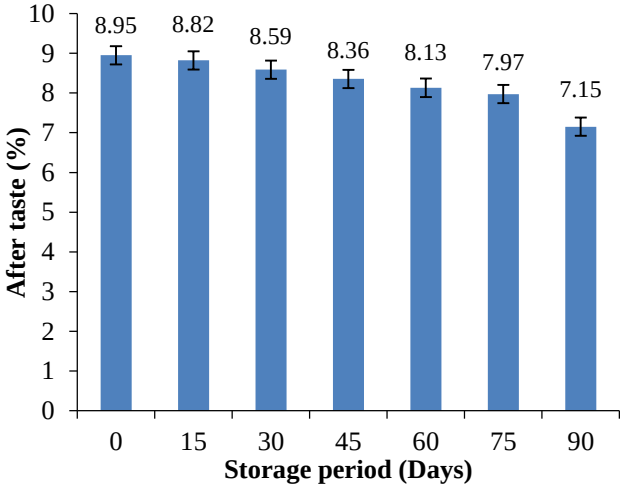

Figure 10. Storage effect on the after taste of sea buckthorn drink.

Overall acceptability: The results indicate that the Overall acceptability of Sea buckthorn was declined during storage. The highest Overall acceptability juice was found at zero day and 15th day in storage, with a mean value of 8.92±0.005 and 8.82±0.006. Whereas, the lowest Overall acceptability of juice was examined at the 90th day and 75th day of storage with the mean of 6.80± 0.040 and 7.97±0.011, as shown in Figure 11.

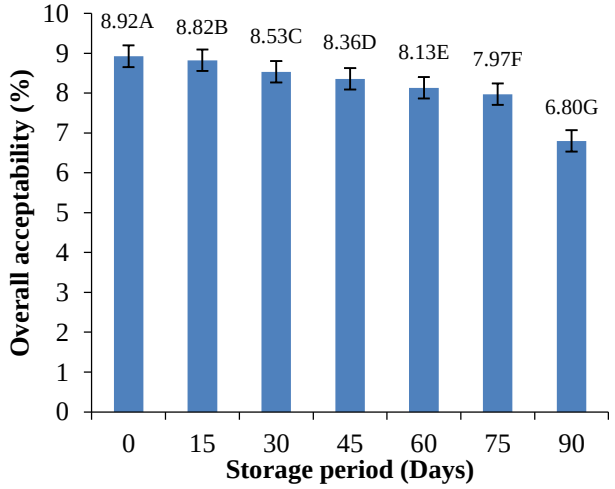

Figure 11. Storage effect on the overall acceptability of sea buckthorn drink.

DISCUSSION

The pH of the sea buckthorn drink decreased during the storage period due to an increase in acidity of the juice. The results of this study are supported by those of Tasnim et al. (2010) and Kumar et al. (2012), who reported that the pH of fruit drinks decreases with storage period due to pectin hydrolysis and the production of free radicals.

The total soluble solids (Brix) of the sea buckthorn drink were increased during the storage period, which might be due to the formation of water-soluble pectin fractions and the formation of mono and disaccharides by the breakdown of polysaccharides. The results are justified with the findings of the person Beveridge et al. (2002), who also reported that the pH of fruit drinks is decreased with storage period due to pectin hydrolysis and production of free radicals

The acidity of the sea buckthorn drink was increased during storage time, which might be due to the formation of free acids by acid hydrolysis of organic acids. The results justify the findings of the individual who further reported that Beveridge et al. (2002) studied the acidity of fruit drinks. In the current study, the sodium content of the Sea buckthorn drink decreased during the storage period. The results are in line with findings by Imran et al. (2010), who also witnessed a decrease in the sodium of the drink during its storage.

In the current study, the calcium of the Sea buckthorn drink decreased during the storage period. These results are in agreement with those of Kumar and Thribhuvan (2014), who reported the decrease in calcium of drink (fruits) during storage. During the study, the potassium decreased during storage in the Sea buckthorn drink. The results in this study are similar to the findings of Pandey et al. (2009), and they also observed a decline in potassium of the drink during the storage period.

Similarly, the color of the sea buckthorn drink was seen to come down in the storage period which might be due to various ingredients and carotenoids present in it. The pH level of fruit drinks also declines with the passage of time due to storage and variation in the amount of ingredients, as reported by Beveridge and Harrison (2001), hence justifying the obtained results. During the storage period, the flavor of the Sea buckthorn drink decreased in the current study. The findings of this study are similar to the results found by Nisperos and Shaw (1990), they also found a decline in the flavor of the drink during storage.

In the present study, the taste of Sea buckthorn drink decreased during the storage time. The results from my study are quite similar to the findings of Selvaraj et al. (1989). The aftertaste of the Sea buckthorn drink decreased during storage time. The results of the study are in accordance with the findings of Cardello (1996), and he also observed a decline in after taste of the drink during storage. The overall acceptability of Sea buckthorn drink decreased during the storage period in the present study. Results are similar to the

results obtained by Mahajan et al. (2009), and they too observed a decrease in overall acceptability of the drink during storage time.

***Conclusion*:** The nutritional and functional characteristics of *Hippophae rhamnoides* L. presented in the current study established it as a promising raw material for the development of health-oriented beverages. The sea buckthorn beverage developed had acceptable physicochemical parameters, a mineral-rich profile, and high antioxidant activity. It was observed that pH, minerals, and sensory scores showed a gradual decline during 90-day storage, though remaining within acceptable limits, thus maintaining the overall quality and consumer acceptability of the product. The formulation with 8 g sea buckthorn powder per 100 mL was optimal, presenting a balanced flavor and acidity, and nutritional profile.

These results confirm that sea buckthorn drink can serve as a stable, safe, and nutritionally enhanced functional beverage with strong market potential. The bioactive ingredients in it contribute not only to nutritional enrichment but also to health promotion by antioxidant and anti-inflammatory mechanisms. Future research should be directed at the optimization of preservation techniques, improvement of shelf life, and further bioavailability and consumer perception studies on a larger scale. Utilizing locally grown sea buckthorn will thus support sustainable food innovation, promote rural economic development, and contribute to improved public health.

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***CRediT author statement*:** Ramsha Akram and Amna Sayal designed and completed the experiments; Muhammad Ishaq prepared the draft; Nida Firdous reviewed and finalized the draft.

***Conflict of Interest*:** The authors declare no conflict of interest.

***Ethical statement*:** This article does not contain any studies regarding humans or animals.

***Availability of data*:** We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

***Consent to participate*:** All authors participated in this research study.

***Informed consent*:** N/A

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***SDGs addressed*:** No poverty, zero hunger, good health and well-being, Industry, Innovation and Infrastructure, etc.

***Policy referred*:** Public Health and Nutrition Improvement Policy; Functional Food and Nutraceutical Development Policy; Food Industry Commercialization and Innovation Policy

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